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Molecular Biology of Beckwith-Wiedemann Syndrome

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Beckwith-Wiedemann syndrome (BWS) is an overgrowth syndrome associated with a predisposition to embryonal tumors, most commonly Wilms' (WT). Overlapping clinical phenotypes are seen in two other disorders, Simpson-Golabi-Behmel syndrome (SGBS) and Perlman syndrome (PS). BWS is a genetically heterogeneous disorder most often associated with normal chromosomes and a negative family history. However, autosomal dominant transmission of BWS is reported, as are chromosome 11p15.5 abnormalities, uniparental paternal disomy (UPD) of chromosome 11p15.5, and altered expression of the imprinted gene insulin-like growth factor 2 (IGF2) from the normally repressed maternal allele. Crucial to our understanding of the large variety of genetic presentations in BWS is the concept of genomic imprinting, a process in which gene expression specific to parent-of-origin is observed.

The current genetic and molecular data for BWS are best explained by a model assuming an imprinted domain for 11p15.5, whereby altered expression of one or more genes in this region contributes to the BWS phenotype. In this model, a defined chromatin structure is reflected in coordinated control of multiple genes in the domain, as well as specific patterns of replication timing and gene expression. Data supporting this viewpoint include the maternally derived 11p15.5 translocation breakpoints associated with BWS, and the recent finding that the normally asynchronous pattern of replication timing for the imprinted gene IGF2 can be disrupted, shifted by a BWS-associated translocation 400 kb from IGF2. As we unravel the molecular basis of the different BWS patient subgroups, we will achieve a better understanding of this overgrowth syndrome and its relationship to WT.

Key words: Beckwith-Wiedemann syndrome, genomic imprinting, chromosome 11p15.5, Wilms' tumor

BECKWITH-WIEDEMANN SYNDROME

Beckwith-Wiedemann syndrome (BWS) represents a defect in human development resulting in a predisposition to cancer [1,2]. The syndrome is characterized by prenatal and postnatal overgrowth, congenital malformations such as omphalocele, and regional overgrowth affecting segmental regions of the body such as arms or legs. It may also involve certain organs or tissues such as the tongue, kidneys, pancreas, adrenal cortex, and liver (Fig. 1). Children with BWS are usually developmentally normal, but are at increased risk for tumor development in the first 5 to 7 years of life. The risk for tumor development is 7.5% in patients without hemihyperplasia, and 12.5% in patients with hemihyperplasia [3,4]. The tumor found with the greatest frequency [3,5] is Wilms' tumor (WT). In the kidneys, nephrogenic rests occur with increased frequency, and are thought to represent persistent blastemal cells that can be precursors of nephroblastoma [6]. Other tumors include hepatoblastoma, rhabdomyosarcoma, neuroblastoma, and adrenocortical carcinoma.

Certain clinical features associated with BWS provide insight into the underlying molecular biology. Phenotypic variability in BWS is significant. Some patients exhibit significant overgrowth, whereas others do not. In addition, the frequency of congenital malformations such as omphalocele correlates poorly with the severity of overgrowth manifestations. Overgrowth sometimes occurs in only one of a pair of organs, e.g., kidney; on one side of the body; or in part of the body. The only positive phenotypic correlation has been that children with hemihyperplasia appear to have an increased incidence of tumor development. No correlation with other clinical findings is reported. This phenotypic variability could be explained by a stochastic model in which there is variable expression of the mutation over time and in different tissues. Alternatively, mutations arising from an unstable constitutional premutation could underlie the development of hemihyperplasia or the increased frequency of tumors. In fact, since most patients with BWS do not develop tumors, further genetic alterations are likely required to produce the tumor phenotype in these patients.

DIFFERENTIAL DIAGNOSIS OF BWS

The existence of other syndromes overlapping phenotypically with BWS such as Perlman syndrome (PS) and Simpson-Golabi-Behmel syndrome (SGBS) indicates a

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Fig. 1. Photographs of a patient with Beckwith-Wiedemann syndrome (left) at 1 month and (right) at 4 years following a partial glossectomy.

common pathway for these developmental processes and the associated predisposition to tumors. PS is characterized by macrosomia, a high neonatal mortality rate, mental retardation, and renal abnormalities including nephroblastomatosis with a high incidence of WT [7–12]. WT associated with PS usually occurs in the first year of life and is often bilateral, in contrast to sporadic WT with a mean age of 35–40 months and only 5% bilaterality. It is not known whether PS is in fact part of the spectrum of BWS or whether it represents a distinct genetic entity, possibly with autosomal recessive inheritance.

SGBS, a recently recognized overgrowth syndrome, shows considerable clinical overlap with BWS [13–22]. SGBS patients exhibit macrosomia, coarse features, cleft lip and/or cleft palate, alveolar ridge grooves, cardiac arrhythmias, intraabdominal visceromegaly, and skeletal anomalies such as vertebral segmentation defects and polydactyly. Developmental delay is a variable feature. An association with WT is documented, although the risk of neoplasia is unknown [23–25] (Hughes-Benzie, personal communication; Terespolsky and Weksberg, unpublished). SGBS is clearly inherited as an X-linked disorder with wide phenotypic variability in affected males and manifesting female heterozygotes. It is mapped to Xq26 [24,26] and therefore genetically distinct from both BWS and PS, which are autosomal.

Hemihyperplasia (HHP) can occur as an isolated finding or in association with a variety of syndromes such as BWS, Proteus syndrome [27], or neurofibromatosis (NF). Isolated forms of HHP are usually sporadic, with an as yet undefined etiology. In a subgroup of cases, HHP may represent a BWS mutation with minimal clinical findings, considering that patients with HHP show a tumor rate of approximately 3.8% [28] and the spectrum of tumors overlaps significantly with those seen with BWS, i.e., WT, hepatoblastoma, adrenocortical carcinoma, neuroblastoma, and adrenal adenoma [4].

This cluster of syndromes points toward a group of genes important in both normal and abnormal development. Constitutional mutation of one or more of these genes may reduce the number of genetic events required for the development of an embryonal tumor such as WT. Knudson's seminal work [29] with respect to two-hit kinetics for tumor development (Fig. 2) must be modified when a gene has been imprinted. In fact, for such genes, "one-hit" kinetics can underlie genetic disease (Fig. 3) and the somatic development of tumors for which imprinted genes are one or more of the targets (Fig. 2). Since the

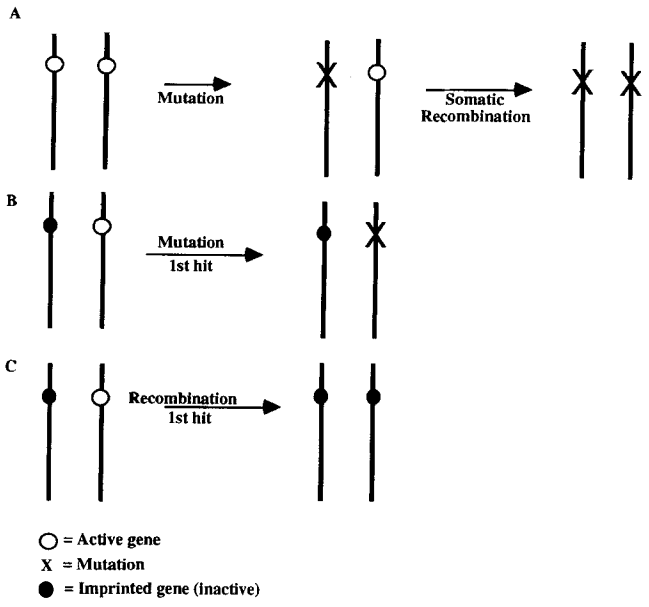

Fig. 2. Kinetics of inactivation of tumor suppressor genes. To inactivate a tumor suppressor gene, where two active alleles are normally present, two hits are required for gene inactivation **(A)**. For an imprinted gene, if only one allele is expressed in an "at risk" tissue, only one hit is required for gene inactivation. This can occur via mutation **(B)** or somatic recombination **(C)**.

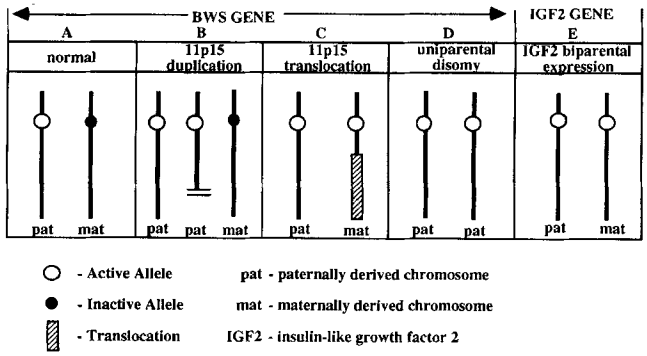

Fig. 3. Etiology of Beckwith-Wiedemann syndrome (BWS). **A:** Normally imprinted BWS locus with the paternal allele active and the maternal allele inactive. **B:** 11p15 duplication with two active paternal BWS alleles and one inactive maternal allele. **C:** 11p15 translocation with the paternal allele active and the maternal allele "cis-activated." **D:** Paternal uniparental disomy (UPD) with two active paternal BWS alleles and no maternal allele. **E:** Biparental origin of biallelic IGF2 expression.

imprinted genes appear to cluster in discrete genomic regions, a single mutation or recombinational event could cause aberrant expression of closely linked genes, both tumor suppressors and growth factors (Fig. 4). In this way, a single genetic event may generate multiple hits in a tumorigenic pathway; subsequently, coordinate dysregulation of the affected genes may be an important cause of predisposition to tumors.

GENETICS AND CYTOGENETICS OF BWS

BWS is itself a genetically heterogeneous disorder. Each genetic subgroup will be discussed in turn, using a reductionist viewpoint to determine what portion of the data can be explained by one imprinted gene or domain on chromosome 11p15. In fact, most of the heterogeneous genetic subgroups of BWS can be rationalized within this model.

Most cases of BWS are sporadic and karyotypically normal. A number of different lines of evidence point to an etiology for BWS on chromosome 11p15 (map Fig. 4) and the involvement of a process known as genomic imprinting. Genomic imprinting refers to a process specific to parent-of-origin whereby the allele derived from one parent (and not the other) can be "marked" and entirely repressed or variably expressed in different tissues. This is shown in Figure 3A where the two normal BWS genes consist of an active paternally derived allele and an inactive maternally derived allele.

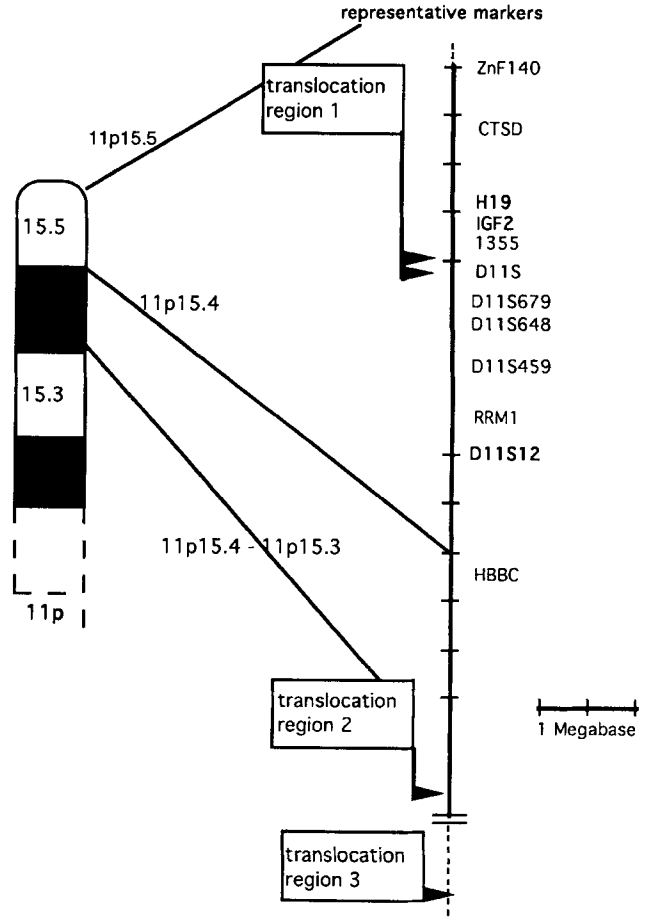

Fig. 4. Map of 11p15 and BWS-associated translocation breakpoint regions.

Autosomal dominant (AD) pedigrees are seen in 10–15% of BWS cases, where linkage of several families to 11p15 has been demonstrated [30,31]. Increased transmission through mothers rather than fathers suggest that genomic imprinting is involved [32]. A small number of experiments on expression of imprinted candidate genes on chromosome 11 have been carried out, but there are no reports of altered expression in these families [33].

Rarely, BWS involves chromosome abnormalities. There are approximately 30 reported cases of chromosome 11 duplications [34–37]. All these duplications include the 11p15 region, but the breakpoints are different between patients, suggesting that the breakpoints do not disrupt a single BWS gene. That is, the phenotype may be due to the presence of an additional gene or genes in the duplicated region. Since these 11p15 duplications are always paternally derived (parent-of-origin effect), the data can be best explained by the presence of an active extra copy of the paternally derived BWS gene [36] (Fig. 3). In BWS, translocations or inversions involving 11p15 are even rarer than duplications [34,38–40]. These BWS-associated translocations have been mapped to three distinct translocation regions on 11p15 using somatic cell hybrids [36] and fluorescence in situ hybridization (FISH) [41,42]. This could mean that there are at least three BWS genes. However, given that all of the translocations associated with BWS are found exclusively on the maternally derived chromosome 11, there is an alternative explanation. Translocations involving other chromosomes may generate long-range cis effects on the imprinted 11p15 region. Experimental evidence in favor of this is presented below. This could result in derepression of the normally imprinted maternal BWS allele (Fig. 3C).

Uniparental paternal disomy (UPD) is a hallmark of sporadic karyotypically normal BWS patients. In paternal UPD, there are two paternally derived chromosome 11p15 regions with no homologous maternal contribution (Fig. 3D). The original description of this finding by Henry et al. [43] has now been confirmed by a variety of different groups [44]. The frequency of UPD for sporadic BWS patients is estimated to be 10–20%. To date, for all patients with BWS the region of UPD has been found to contain most commonly the insulin IGF2 region. No patients have been found to carry two different homologous paternally derived chromosomes, while all UPD patients exhibit somatic mosaicism. Mechanistically, this means that the UPD has arisen from a somatic recombination event. Therefore, it is not known what the true frequency of UPD is in sporadic BWS cases, since most somatic tissues are inaccessible for testing. The somatic recombination event generating UPD in BWS is the same as one of the mechanisms shown to generate loss of heterozygosity (LOH) in WT and other tumors associated with BWS (Fig. 5E). A correlation has been suggested between the finding of UPD and increased tumor risk above that for other BWS patients, but there is currently insufficient data to support this view.

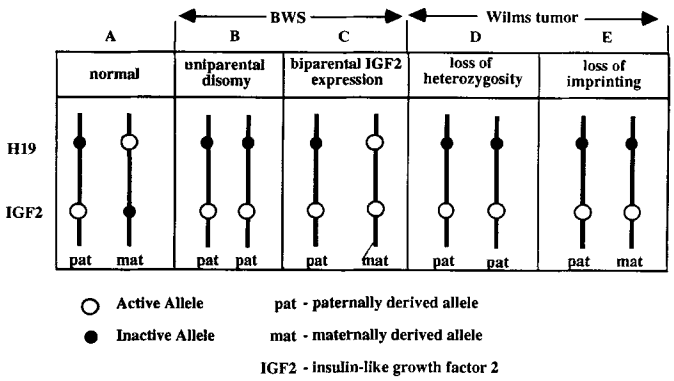

Fig. 5. Expression of 11p15 imprinted genes, IGF2 and H19, in Beckwith-Wiedemann syndrome and Wilms tumor. **A:** Normally imprinted 11p15 region with the paternal IGF2 allele active and the maternal allele inactive. The paternal H19 is inactive and the maternal H19 allele is active. **B:** Paternal uniparental disomy (UPD) with 2 active IGF2 alleles and 2 inactive H19 alleles. **C:** Biparental origin of biallelic IGF2 expression with normal maternal monoallelic H19 expression in BWS. **D:** Loss of heterozygosity in Wilms tumor is associated with loss of the maternal 11p15 chromosome region so that there are two paternally derived contributions with two active IGF2 alleles and two inactive H19 alleles. **E:**"Loss of imprinting" in Wilms' tumor is associated with biallelic expression of IGF2 and loss of H19 expression.

INSULIN-LIKE GROWTH FACTOR 2 AND BWS

Insulin-like growth factor 2 (IGF2) is a human growth factor [45]. The gene for IGF2 has been mapped to 11p15.5 and is imprinted with monoallelic expression of the paternally derived allele in both mouse and human [46–48]. These findings for IGF2, its map position, its function, and an imprinting pattern involving monoallelic expression of the paternal gene make it a good candidate gene for BWS. Furthermore, the pattern of expression of IGF2, both anatomically and developmentally, coincides with abnormalities associated with the BWS phenotype, i.e., general somatic overgrowth late in pregnancy and early childhood; overgrowth of specific organs such as kidney, liver, adrenal cortex, and pancreas; and abnormalities in IGF2 expression in tumors associated with BWS.

IGF2 is only one of several imprinted genes in the 11p15.5 region. A second imprinted gene, H19, transcribed into a functional RNA but not translated, may function as a tumor suppressor gene [49]. H19 maps 200 Kb distal to IGF2 [50]. Recent studies in mouse support a model in which there is coordinate regulation of H19 and IGF2. Leighton et al. [51] recently demonstrated that mice lacking the H19 gene product show biallelic IGF2 expression as predicted by the enhancer-competition model of Tighlman [52]. These mice are 30% heavier than their counterparts, presumably on the basis of in-

creased IGF2 expression rather than lack of H19 expression.

Relaxation of IGF2 imprinting occurs in about 50% of WT, the embryonal tumour most commonly seen in BWS. In a substantial number of tumors exhibiting biallelic IGF2 expression, H19 gene expression is repressed [53,54], consistent with the enhancer-competition model for the regulation of these two genes. Studies of IGF2 and H19 expression in BWS have also been carried out. For IGF2, altered expression of the maternal allele was seen in 4 out of 6 fibroblasts and 1 tongue tissue in selected patients; i.e., patients were sporadic, karyotypically normal, and were not found to have UPD. Interestingly, the H19 expression pattern in these skin fibroblasts is not consistent with the WT profile, nor with the enhancer competition model in that monoallelic maternal H19 expression is maintained even in BWS skin fibroblasts that exhibit biallelic IGF2 expression (Fig. 5C).

IMPRINTING AND DNA METHYLATION

Gene regulation in imprinted regions of the genome is poorly understood. What is clear is that DNA methylation, chromatin structure, and domain-like regions all play a role. The idea of an imprinted domain was first proposed to explain the human diseases Prader-Willi syndrome (PWS) and Angelman syndrome (AS) where gene expression and methylation patterns of multiple genes seem to be regulated by an "imprinting control element" [55]. DNA methylation is known to be involved in the regulation of gene expression during development. Methyl moieties at specific CpG residues suppress transcription by a cis-acting mechanism affecting DNA-protein interactions, thus altering the accessibility of genes to transacting factors in the cell. It is probable that one of two allelic residues will be associated with the imprinting "mark." For some imprinted genes, the "mark" involves hypomethylation, whereas for other genes it is associated with hypermethylation. Associated with these allelic methylation differences are differences in transcription of the genes that are known to be tissue-specific and often dependent on developmental stage.

METHYLATION STUDIES OF 11p15 IN BWS

Studies of methylation of 11p15 imprinted genes show that methylation imprints characteristic of repressed or active states are detectable for both the H19 and IGF2 genes (Fig. 6). Normally in methylase deficient mice, where methylation associated imprints cannot be maintained, the expression of IGF2 and H19 is altered [56]. Similarly, altered expression of IGF2 and H19 in WT reflects altered methylation patterns [53,57,58]. In BWS patients, a characteristic paternally derived methylation pattern is seen only in leukocytes from patients with UPD [59,60], but altered methylation does not appear to be a general characteristic. Furthermore, even in skin fibroblasts with biallelic IGF2 expression, consistent IGF2 methylation alterations are not seen (Squire and Weksberg, unpublished). This is of interest, since the normal H19 expression is maintained, supporting the concept that H19 is the primary imprint in this domain.

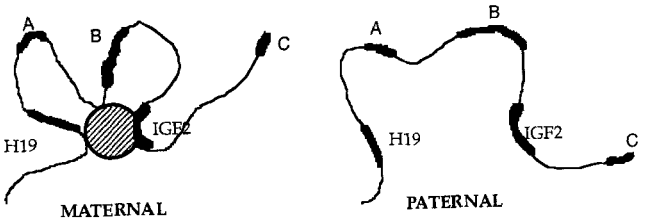

Fig. 6. Differential DNA methylation associated with different chromatin contexts for paternally and maternally derived 11p15 regions.

IMPRINTING, CHROMATIN STRUCTURE, AND DNA

Studies of X chromosome inactivation in females indicate that late replication is strongly associated with transcriptional inactivity, methylation, and changes in chromatin structure. In general, this pattern holds for autosomal imprinted regions in the genome as well, with expressed loci being early replicating and the silent (imprinted) loci late replicating [61]. It is speculated that late replication is a consequence of the chromatin being "less accessible" to DNA binding proteins and factors mediating DNA replication and transcription. Actively transcribing genes are replicated early in S phase, which may allow them to form associations with free transacting factors and become transcriptionally competent. For genes replicating later in the cell cycle, such factors will be depleted, resulting in transcriptional silence throughout the cell cycle. It is presently unclear whether transcriptional inactivity leads to retarded replication timing, or vice versa. Nevertheless, there is a general correlation between transcriptional competence and early replication; when one allele has retarded replication, it is inactive and the locus is probably imprinted. The position of replication origins and local chromosomal context are likely highly pertinent to the overall mechanism of genomic imprinting [62].

REPLICATION TIMING OF IGF2 IN BWS

Chromosomal context has been suggested to profoundly influence replication timing and the activity of DNA replication origins [62]. To determine whether long-range chromatin effects do occur in the imprinted 11p15 region in BWS patients, we elected to assess replication timing of the imprinted gene IGF2 in BWS patients carrying translocations as far as 400 Kb away from this particular gene. An asynchronous pattern of replication timing, i.e., 25–35% asynchrony, normally seen with an

imprinted gene such as IGF2, was in fact seen in the controls. However, the translocation 11;22 mapping 400 kb away from IGF2 [41] resulted in partial loss of asynchrony of IGF2 replication, indicating a long-range effect on the imprinted domain as measured by replication timing. The BWS patient with the 11;22 translocation showed a trend towards loss of asynchronous replication when compared with controls. This trend has been found to be statistically significant in two separate experiments. Since the 11;22 BWS translocation contains 400 kb of DNA between the translocation and IGF2, loss of asynchronous replication might occur because long segments of normal chromatin structure are required for normal regulation and transcription in this region. That is, the introduction of non-chromosome 11 DNA into this region might cause the translocated region to be affected by the cis effects of chromosome 22, which may cause changes in conformation and thereby affect the timing of replication. Since there are approximately 10 such 11p15 translocations spanning over 1 megabase of DNA and involving many other chromosomes [42], we propose that DNA rearrangements that place 11p15.5 near other regions of the genome may disrupt putative chromatin control elements.

TWINS DISCORDANT FOR BWS

Among the intriguing observations of clinical phenotypes associated with BWS are reports of approximately 20 monozygotic (MZ) twin pairs, most of whom are female and discordant for BWS. In addition, one concordant female pair has been described, one concordant male pair and two discordant male pairs. Of particular interest in this regard is the possibility of a postzygotic somatic recombination to explain the discordance in MZ twins [63]. Skin fibroblasts derived from three such twin pairs were examined, since the blood of MZ twins often undergoes mixing in utero and may not reflect the genetic constitution of only one twin. All pairs were tested for monozygosity at multiple loci and found to have a >99.9% probability of being MZ. However, restriction fragment length polymorphism (RFLP) studies to compare 11p15 contributions in BWS discordant twin pairs show no differences between these three twin pairs. In this way, paternal isodisomy for the IGF2/insulin region was shown to be an unlikely explanation for the discordance, since somatic mosaicism cannot be entirely excluded via a complete tissue survey in these twins.

An alternate explanation of the unusual female MZ twin discordance in BWS relates to possible differences in X chromosome inactivation between twins. This type of discrepancy has been described for X-linked disorders such as Duchenne muscular dystrophy where two MZ female twins both carrying the Duchenne muscular dystrophy (DMD) mutation showed opposite phenotypes; i.e., one expressed the DMD phenotype and the other was normal [64]. This difference in phenotype is due to opposite skewing of X inactivation: in the affected twin the X chromosome carrying the normal DMD allele is almost exclusively inactivated, where in the normal twin the X chromosome carrying the DMD mutation is almost exclusively inactivated. For this reason, and because the X-linked overgrowth syndrome SGBS overlaps significantly with BWS, we assessed patterns of X-inactivation in fibroblasts of two MZ female twin pairs discordant for BWS to determine whether they had differential skewing of the X chromosome. In fact, in one female twin pair, both showed skewed X-inactivation, but in the same direction, while in another twin pair very little skewing was observed. Thus, differences in X inactivation patterns between MZ twins is not a general explanation for the occurrence of such twins. Other mechanisms that may explain the BWS discordance of MZ twins include as yet unidentified X chromosome or autosomal differences between the twins, or differential gene expression/imprinting of loci in early development, when the rate of development of females lags behind that of males.

To determine whether X inactivation plays a role in the etiology of sporadic BWS, Bird et al. [65] have examined a population of BWS individuals to determine whether they have skewed X inactivation different from that of the general population. Their positive findings support the concept that the X chromosome is involved in the expression of the BWS phenotype, at least in some patients, either directly, via the X-linked SGBS, or via other genes which may control imprinting of autosomal loci.

CONCLUSIONS

Recent results suggest the existence of chromosome domains containing clusters of imprinted genes that may be controlled in a regional manner. Therefore, it seems likely that the regulation of imprinted genes is complex, utilizing multiple control mechanisms and most certainly involving both local and regional effectors of expression. Our knowledge of the basis of BWS and its associated tumors is still rudimentary, but it is clear that a number of powerful new experimental tools have become available to clarify the basis of overgrowth syndromes and the associated tumors in the near future.

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COMMENTARY

Weksberg and Squire discuss the genetics, cytogenetics and molecular genetics of Beckwith-Wiedemann syndrome (BWS). The BWS gene is thought to be localized on chromosome 11p15, a locus also believed to harbor the second Wilms' tumor suppressor gene, *WT2*. With this in mind, the authors indicate that the available data for BWS are best explained by a model assuming an imprinted domain for chromosome 11p15, whereby altered expression of one or more genes in this region contribute to the BWS phenotype. For an explanation of the term, "imprinting," see the manuscript by Moulton et al., in this issue.